Following these modifications, a new accuracy and precision study was carried out on Dasanit 15% granular formulations which were quantitatively prepared in the laboratory by a spray coating technique, simulating commercial production. Three riffler fractions of each preparation were analyzed on three different days. As shown in Table IV, the mean value for each preparation was in very good agreement with the theory and the σ values for accuracy and precision were identical. All of these samples as well as the secondary standard were stirred prior to sampling, while the samples shown in Table I were not. A comparison of the σ values for Dasanit granules showed that utilizing a secondary standard and introducing the standardized stirring step halved the confidence limits.

LITERATURE CITED

Detergents and Emulsifiers Annual, 29 (1963).
Dixon, W. J., *Biometrics* **9**, 74 (1953).
Whitley, R. W., Alburn, H. E., *Ann. N.Y. Acad. Sci.* **130**(2), 634 (1965).

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Residue Study of Phenoxy Herbicides in Milk and Cream

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Cows were fed a complete ration containing 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 2-(2,4,5-trichlorophenoxy)propionic acid, or 2-methyl-4-chlorophenoxyacetic acid at six levels from 10 to 1000 ppm for 2 or 3 weeks at each level. Milk and cream samples were collected at predetermined intervals during the feeding of these chemicals and for 7 days following withdrawal of the highest level. Residues of the acids and their phenol moieties were extracted with diethyl ether, separated by liquid chromatography on alumina, and determined as esters and phenols by electron capture or microcoulometric gas chromatography. The procedure was used to quantitate the chemicals down to 0.05 ppm, with overall average recoveries of greater than 80%. The average residues found in milk at the highest feeding level were: 0.06 ppm, 2,4-dichlorophenoxyacetic acid; <0.05 ppm, 2,4-dichlorophenol; 0.42 ppm, 2,4,5-trichlorophenoxyacetic acid; 0.23 ppm, 2,4,5-trichlorophenol; 0.12 ppm, silvex; <0.05 ppm, 2,4,5-trichlorophenol; and <0.05 ppm, 2-methyl-4-chlorophenoxyacetic acid; 0.06 ppm, 2-methyl-4-chlorophenol. Residues of all chemicals decreased rapidly upon removal of the chemicals from the feed.

Work at Cornell University (Gutenmann *et al.*, 1963a,b; Bache *et al.*, 1964; St John *et al.*, 1964) indicated that ingested phenoxy compounds are not readily transferred to milk. Doses of various compounds administered daily to individual cows at rates equivalent to 5 ppm in 50 lb of feed per day for up to 5 days produced no detectable residues in milk.

Residues up to 0.06 ppm of 2,4-D (2,4-dichlorophenoxyacetic acid) were found in milk from cows grazed on pastures sprayed with isopropyl or isooctyl esters of 2,4-D at 2 lb of acid equivalent per acre (Klingman *et al.*, 1966).

This study was designed to determine the residue levels of 2,4-D, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), silvex [2-(2,4,5-trichlorophenoxy)propionic acid], or MCPA (2-methyl-4-chlorophenoxyacetic acid) and their corresponding phenol moieties which might occur in milk and cream when cows were fed very high levels of phenoxy herbicides for prolonged periods of time.

APPARATUS

Barber-Colman Models 10 and 5000 gas chromatographs equipped with Sr^{90} electron capture detectors were used for determining 2,4-D, 2,4,5-T, and silvex and their corresponding phenols. The Infotronics GTS-20 system for microcoulometric determination of halogen was used for MCPA and 2-methyl-4-chlorophenol.

Residue Research, Ag-Organics Department, Dow Chemical U.S.A., Midland, Michigan 48640.

REAGENTS

Analytical grade 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2-methyl-4-chlorophenol, 2,4-D, 2,4,5-T, silvex, MCPA, and the methyl esters of the four phenoxy acids used in this study are obtainable from the Sampling Coordinator, Ag-Organics Department, Dow Chemical U.S.A.

GAS CHROMATOGRAPHIC OPERATING CONDITIONS

The electron capture systems consisted of 185-cm × 3-mm i.d., U-shaped borosilicate glass columns packed with 4% LAC-446 + 0.55% H_3PO_4 on 80–100 mesh Chromosorb W-HP. Chromatograph oven temperatures adjusted to give a retention time of 2.5 to 3.5 min for all chemicals were: 150°C for 2,4-dichlorophenol; 180°C for 2,4,5-trichlorophenol and the methyl ester of silvex; and 190°C for the methyl esters of 2,4-D and 2,4,5-T. Detector and injector block temperatures were 220°C. The carrier gas was prepurified nitrogen with a flow rate of approximately 100 cm^3/min. Injection volume was 2 μl.

The microcoulometric system included a quartz pyrolysis furnace, C-200-AR microcoulometer, T-300-S titration cell, and R-100-I strip chart recorder. A capillary stainless steel line wrapped with heater tape was used to connect the furnace to the gas chromatographic column in a Barber-Colman Model 5000 oven. The 185-cm × 6-mm i.d., U-shaped borosilicate glass column was packed with 6% DC-200 on Gas Chrom Q, 80–100 mesh. A "T" joint was fabricated on the outlet end of the column with one side connected to the vent and the other to the furnace. The column was operated at

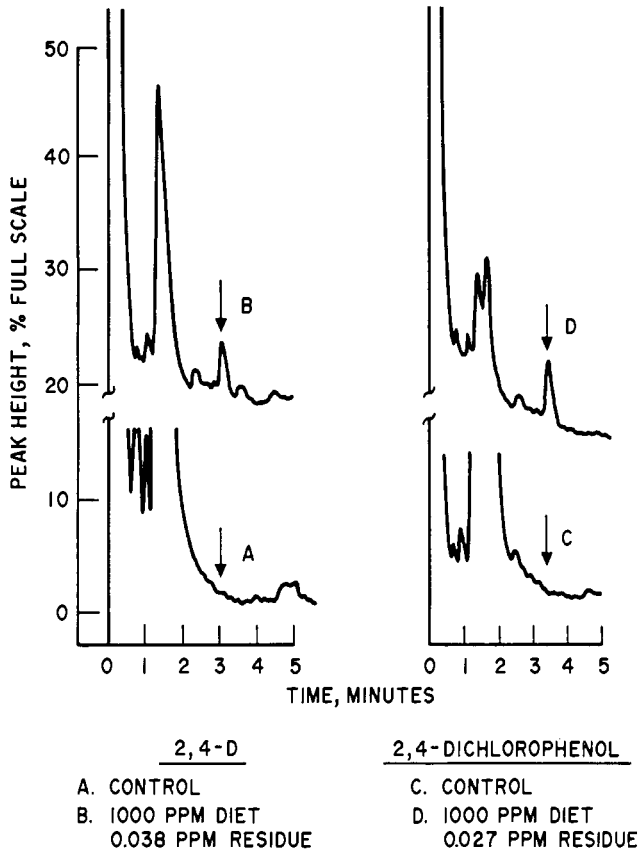

Figure 1. Typical chromatograms from determination of 2,4-D and 2,4-dichlorophenol in milk

170°C for 2-methyl-4-chlorophenol and 210°C for the methyl ester of MCPA, with the injection block at 225°C and transfer line at 220°C. Furnace temperatures were: inlet, 220°C; center and outlet, 900°C. Other settings were: bias voltage, 260 mV; gain, 200; and range, 500 ohm. Oxygen flow rate was 70 cm^3/min and nitrogen carrier gas flow was approximately 78 cm^3/min. Injection volume was 20 μl.

EXPERIMENTAL PROCEDURES

Holstein cows were conditioned for 2 weeks on a complete dairy ration. A total of 36 lb of feed was given to each cow daily, half at each milking. Three cows were maintained on this feed as controls and three cows were switched to dairy ration containing the respective phenoxy acid. The fortified feeds were prepared by blending 10 to 25% (w/w) concentrates of each chemical on silica gel with feed to make rations containing 10, 30, 100, 300, or 1000 ppm of chemical in the feed. Commercial production lots of the four acids were used.

For each chemical, three cows were fed 14 days at each of the low levels, 21 days at 1000 ppm, and then 7 days on feed containing no chemical. Milk samples were collected from each cow at predetermined intervals by combining $^1/_2$ pint from the evening milking with an equal amount collected the following morning. Six samples were collected during each 2-week period, nine samples during the 3-week period, and four samples during the withdrawal period.

Cream collected from morning milk only was obtained by compositing the milk from the three test cows and separating on a DeLaval Model 100 electric farm separator adjusted to give medium heavy cream.

Samples were stored frozen in 1-pt tin cans until analyzed. Analysis was begun with samples from the 1000-ppm level and proceeded to lower levels until residues from all cows in a group were below 0.05 ppm. A total of 422 samples were analyzed in this study, excluding recovery determinations.

Animal weight and milk production records were kept. No adverse effect due to ingestion of the chemicals was noted in any of the animals.

ANALYTICAL METHODS

2,4-D and 2,4-Dichlorophenol in Milk. The following procedure was used. Pipet 10 g of well mixed milk into a 12-dram, 25 × 100 mm vial. Add 1 ml of concentrated H_2SO_4, and place on top of a steam bath for 30 min. Cool and add 2 g of NaCl and 15 ml of diethyl ether. Cap the vial with a cap with a Polyseal liner, shake it for 5 min on a mechanical shaker, and centrifuge. Place about 1 g of Woelm acidic alumina, activity grade I, in a 17-cm × 10-mm i.d. liquid chromatographic tube having a coarse-sintered glass disk. Transfer the ether phase to the alumina column, collecting the effluent in a clean 12-dram vial. Re-extract the milk by shaking it with another 10 ml of diethyl ether and transfer the ether to the column. Wash the column with 10 ml of diethyl ether.

Add 5 ml of 1 *N* NaOH to the combined column effluent, cap the vial, and shake it for 3 min and centrifuge. Draw off the ether layer and discard. Direct a stream of air on the remaining aqueous phase to remove ether. Add 0.5 ml of concentrated H_3PO_4, 1 g of NaCl, and 10 ml of benzene to the vial. Cap, shake for 3 min, and centrifuge. Inject 2 μl of the benzene phase into the gas chromatograph to determine 2,4-dichlorophenol.

Elute the alumina column with 20 ml of 0.25% $NaHCO_3$ solution, collecting the eluate in a clean 12-dram vial. Acidify the solution with 0.5 ml of concentrated H_3PO_4 and add about 1 g of NaCl. Successively extract this solution with two 5-ml portions of diethyl ether. Transfer each extract to a 10-ml volumetric flask containing 1 ml of benzene and heat each on a steam bath to remove the ether. Add 0.5 ml of diazomethane reagent (DeBoer and Backer, 1963). Allow to stand for 3 min, then add a boiling chip and remove the ether from the solution by heating on a steam bath. Dilute to volume with benzene and inject 2 μl into the gas chromatograph to determine 2,4-D.

Typical chromatograms for 2,4-D and 2,4-dichlorophenol analyses in milk are shown in Figure 1. The chromatograms from the 2,4,5-T and silvex analysis were similar to these.

2,4,5-T and 2,4,5-Trichlorophenol in Milk. This procedure differed only slightly from that described above. Collect the diethyl ether effluent from the alumina column in a 50-ml graduated mixing cylinder and dilute with ether to 50 ml. Inject this solution into the gas chromatograph to determine 2,4,5-trichlorophenol.

Equilibrate the acidified alumina column eluate with 10 ml of diethyl ether by shaking for 5 min. Centrifuge and pipet 5 ml of the ether layer into a 10-ml volumetric flask for esterification.

Silvex and 2,4,5-Trichlorophenol in Milk. This procedure was the same as that for 2,4,5-T and 2,4,5-trichlorophenol, with the following exceptions. Heat the milk with 1 ml of concentrated H_3PO_4, add 4 g of NaCl, and use two additional 10-ml portions of diethyl ether for extraction.

MCPA and 2-Methyl-4-chlorophenol in Milk. The following procedure was used. Add 1 ml of concentrated H_3PO_4 and 4 g of NaCl to 20 g of milk and heat the mixture on a steam bath. Extract with two 15-ml and two 10-ml portions of diethyl ether as described for 2,4-D.

Table I. Recovery of Phenoxy Acids and Phenols Added to Milk and Cream over the Range from 0.05 to 1.0 ppm

Chemical	Milk			Cream		
	Number of determinations	Recovery, %		Number of determinations	Recovery, %	
		Range	Avg		Range	Avg
2,4-D	8	90–100	95	8	84–100	93
2,4-Dichlorophenol	8	80–96	90	8	80–98	88
2,4,5-T	13	80–100	92	9	85–94	90
2,4,5-Trichlorophenol	10	85–92	89	10	80–92	88
Silvex	21	86–98	90	16	86–96	91
2,4,5-Trichlorophenol	21	78–106	93	16	88–98	94
MCPA	26	80–120	100	17	70–110	89
2-Methyl-4-chlorophenol	27	68–110	85	16	58–98	79

Table II. Residues of 2,4-D and 2,4-Dichlorophenol in Milk and Cream from Cows Fed Ration Containing 1000 ppm of 2,4-D

Days on diet	Residue of 2,4-D, ppm				Residue of dichlorophenol, ppm			
	Milk Cow no.			Cream composite	Milk Cow no.			Cream composite
	22	7	12		22	7	12	
3	0.05	0.06	<0.05	...	<0.05	0.06	0.05	...
10	<0.05	0.08	<0.05	...	<0.05	0.06	<0.05	...
17	0.05	0.11	0.05	0.12	<0.05	0.05	<0.05	<0.05
18	0.05	0.12	<0.05	<0.05	<0.05	0.06	<0.05	<0.05
19	0.05	0.09	<0.05	0.05	<0.05	0.06	<0.05	<0.05
20	0.06	0.12	<0.05	0.06	0.08	0.06	<0.05	<0.05
21	<0.05	0.07	<0.05	<0.05	<0.05	0.05	<0.05	<0.05

Table III. Residues of 2,4,5-T and 2,4,5-Trichlorophenol in Milk and Cream from Cows Fed 2,4,5-T

ppm 2,4,5-T in diet	Days on diet	Residue of 2,4,5-T, ppm				Residue of trichlorophenol, ppm			
		Milk Cow no.			Cream composite	Milk Cow no.			Cream composite
		36	7417	30		36	7417	30	
100	2	<0.05	<0.05	<0.05	...	...	...	...	...
	5	<0.05	<0.05	<0.05	...	0.06	0.05	<0.05	...
	9	<0.05	<0.05	<0.05	...	...	...	...	...
	10	<0.05	<0.05	<0.05	<0.05	...	...	...	0.05
	11	<0.05	<0.05	<0.05	...	...	...	...	...
	12	<0.05	<0.05	<0.05	<0.05	0.05	0.07	<0.05	0.06
300	2	0.08	0.08	0.20	...	0.08	0.12	<0.05	...
	5	<0.05	<0.05	0.08	...	...	...	...	...
	9	0.05	0.06	0.28	0.10	...	...	...	0.09
	10	0.06	0.07	0.31	0.08	...	...	...	0.10
	11	<0.05	0.05	0.13	<0.05	0.09	0.13	0.16	0.12
1000	2	0.31	0.26	0.78	...	0.16	0.37	0.39	...
	5	0.44	0.27	0.54	...	...	...	...	...
	9	0.42	0.32	0.44	...	...	...	...	...
	12	0.37	0.30	0.29	...	0.17	0.21	0.22	...
	16	0.23	0.36	1.0	0.41	0.15	0.31	0.23	0.21
	17	0.33	0.28	0.75	0.25	0.18	0.23	0.21	0.17
	18	0.49	0.29	0.38	0.17	...	...	...	0.20
	19	0.33	0.40	0.35	0.27	...	...	...	0.20
	20	0.23	0.28	0.32	0.21	0.15	0.23	0.25	0.18
0	1	0.07	0.12	0.12	...	0.09	0.22	0.13	...
	3	<0.05	<0.05	<0.05	...	<0.05	<0.05	<0.05	...
	5	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	7	<0.05	<0.05	<0.05	...	<0.05	<0.05	<0.05	...

Put the ether extract through a 1-g column of alumina. Collect the ether effluent in a beaker and evaporate to about 1 ml on a steam bath. Transfer the contents of the beaker to a 12-dram vial using about 10 ml of hexane, and add hexane to bring the volume to about 15 ml. Add 10 ml of 1 *M* NaOH, cap the vial, and shake it for 5 min. Centrifuge and draw off the hexane layer and discard. Direct a stream of air on the remaining aqueous phase to evaporate any remaining hexane. Acidify with 1 ml of concentrated H_3PO_4 and add 4 g of NaCl. Pipet 2 ml of benzene into the vial, cap, and shake it 5 min, and centrifuge. Inject 20 μl of the benzene phase into the gas chromatograph to determine 2-methyl-4-chlorophenol.

Equilibrate the acidified alumina column eluate with 3 ml of diethyl ether by shaking for 5 min. Centrifuge and transfer 2 ml of the ether layer to a 2-ml volumetric flask. Evaporate the ether solution to about 1 ml with a stream of air. Add about 0.5 ml of benzene and continue the evaporation to a volume of about 0.5 ml. Esterify with 1 ml of diazomethane reagent using the procedure described for 2,4-D. Dilute to volume with benzene and inject 20 μl into the gas chromatograph to determine MCPA.

Table IV. Residues of Silvex in Milk and Cream from Cows Fed Ration Containing 1000 ppm of Silvex

Days on diet	Residue of silvex, ppm: Milk Cow no. 96	Milk Cow no. 90	Milk Cow no. 9078	Cream composite
3	0.06	0.05	0.12	...
6	0.06	0.12	0.10	...
10	0.07	0.06	0.14	...
13	0.05	0.09	0.14	...
17	0.08	0.08	0.18	0.16
18	0.08	0.06	0.18	0.16
19	0.05	0.11	0.14	0.14
20	0.15	0.12	0.19	0.13
21	0.11	0.09	0.23	0.20

Analysis of Cream. The procedures for cream were very similar to those for milk. Dilute cream samples with 5 ml of water before heating with acid on the steam bath. Use one additional 15-ml portion of diethyl ether to extract cream for 2,4-D, 2,4,5-T, silvex, and the corresponding phenol determinations.

Partition the first ether extract with 10 ml of 1 *N* NaOH solution, and discard the ether. Acidify the aqueous phase and extract it with the second 15-ml ether extract of cream. This partition removed much of the fat before the ether extract was put on the alumina column.

For MCPA and 2-methyl-4-chlorophenol, extract with two 12-ml portions of hexane. Combine and partition with 10 ml of 1 *N* NaOH. Discard the hexane and acidify the aqueous layer. Then extract the cream and this acidified aqueous layer successively with three 10-ml portions of diethyl ether. After the three 10-ml ether extracts have run through the alumina column, wash it with an additional 12 ml of ether.

The remainder of the procedures followed that for milk.

Standard Curves. Standard solutions of the four methyl esters and three phenols were prepared in benzene and used to determine response curves. Peak heights were plotted *vs.* acid equivalent or phenol concentrations. These curves were prepared daily and checked repeatedly during the day. A working concentration range giving linear or near-linear response was obtained for all compounds except the methyl ester of 2,4-D. The quantities of standards injected were: 2,4-D, 0.1 to 1.0 ng; 2,4,5-T, 0.02 to 0.2 ng; silvex, 0.01 to 0.1 ng; and MCPA, 4 to 40 ng. Similar amounts were injected for the corresponding phenols.

Calculations. Quantitation was done using peak heights and the standard curves.

RESULTS AND DISCUSSION

No significant blanks were found in a total of 102 control samples analyzed by the procedures described. The efficiencies of the methods were determined by fortifying control milk and cream with known amounts of compounds and applying the analytical procedures (Table I).

No residues of 2,4-D or 2,4-dichlorophenol greater than 0.05 ppm were found in milk or cream at the 300-ppm or lower feeding levels. An average of 0.06 ppm of 2,4-D and <0.05 ppm of 2,4-dichlorophenol was found in milk from the 1000-ppm feeding level (Table II). No residues greater than 0.05 ppm were found in any of the samples taken during the withdrawal period.

No residues of 2,4,5-T or 2,4,5-trichlorophenol greater than 0.05 ppm were found at the 30- to 10-ppm feeding levels. At the 1000-ppm feeding level, average residues found were 0.42 ppm of 2,4,5-T in milk and 0.26 ppm in cream, and 0.23 ppm of 2,4,5-trichlorophenol in milk and 0.19 ppm in cream (Table III).

Residues of silvex were found only in the 1000-ppm feeding level where the average was 0.12 ppm in milk and 0.16 ppm in cream (Table IV). No residues of 2,4,5-trichlorophenol were found in any of the samples of milk or cream from cows fed silvex.

Residues of MCPA greater than 0.05 ppm were found in only six samples of milk from cows fed at the 1000-ppm level (Table V). Small residues of 2-methyl-4-chlorophenol were found in milk at the 300- and 1000-ppm feeding levels and in cream at the 1000-ppm feeding level.

Withdrawal of the chemicals from the diet resulted in rapid disappearance of residues from the milk. Where residues greater than 0.05 ppm were found, the residues dropped below 0.05 ppm on the first day after withdrawal of 2,4-D, silvex, and MCPA from the diet and below 0.05 ppm on the third day after withdrawal of 2,4,5-T from the diet.

No significant difference was found between residues in milk and cream.

Good agreement was found among the residues within the groups of cows at all feeding levels of all chemicals. This is

Table V. Residues of MCPA and 2-Methyl-4-chlorophenol in Milk and Cream from Cows Fed MCPA

ppm MCPA in diet	Days on diet	Residue of MCPA, ppm: Milk Cow no. 22	Milk Cow no. 7	Milk Cow no. 12–36[a]	Cream composite	Residue of 2-methyl-4-chlorophenol, ppm: Milk Cow no. 22	Milk Cow no. 7	Milk Cow no. 12–36[a]	Cream composite
300	3	<0.05	<0.05	<0.05	...	<0.05	<0.05	<0.05	...
	6	<0.05	<0.05	<0.05	...	<0.05	<0.05	<0.05	...
	10	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.06	<0.05
	11	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.08	<0.05
	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.06	<0.05
	13	<0.05	<0.05	<0.05	<0.05	<0.05	0.06	0.08	<0.05
1000	3	0.06	<0.05	<0.05	...	<0.05	0.09	<0.05	...
	6	<0.05	<0.05	<0.05	...	<0.05	0.11	<0.05	...
	10	<0.05	<0.05	<0.05	...	<0.05	0.08	<0.05	...
	13	0.06	<0.05	<0.05	...	<0.05	0.12	<0.05	...
	17	<0.05	<0.05	<0.05	<0.05	0.08	0.08	<0.05	0.08
	18	<0.05	<0.05	0.06	<0.05	0.05	0.15	<0.05	0.09
	19	<0.05	<0.05	0.05	<0.05	0.08	0.16	<0.05	0.08
	20	<0.05	<0.05	0.07	<0.05	0.08	0.17	<0.05	0.05
	21	<0.05	0.06	<0.05	...	0.08	0.15	<0.05	...

[a] Cow no. 12 was replaced by cow 36 after the end of the 300-ppm MCPA regimen.

Table VI. Residues of 2,4,5-T and 2,4,5-Trichlorophenol in Milk Determined by Procedures with and without Acid Hydrolysis

2,4,5-T in diet, ppm	Residues found, ppm	
	Hydrolyzed	Not hydrolyzed
	2,4,5-T	
0	0	0
300	0.19	0.17
300	0.07	0.09
300	0.26	0.22
1000	0.26	0.27
	2,4,5-Trichlorophenol	
1000	0.19	0.20
1000	0.21	0.18

best indicated in Table III for 2,4,5-T fed at 1000 ppm. Daily variation of results for a single cow, No. 30, was as much as 0.29–1.0 ppm, which was as great as variation between cows on a given day, *e.g.*, 0.23–1.0 ppm on day 16.

Residues increased as chemical feeding rates increased. At all feeding rates residues had reached a plateau by the time sampling began on the second or third day. At the highest feeding level, phenoxy acid residues in milk ranged from approximately <0.005 to 0.04% of the concentration in the diet for the four compounds. This is similar to the 0.02% found for 4-amino-3,5,6-trichloropicolinic acid (Kutschinski, 1969).

The acid hydrolysis procedure was used for all samples only because it facilitated separation of the phases by centrifugation during the extractions with diethyl ether. Some milk samples from the 2,4,5-T experiment were prepared without the acid hydrolysis to check the possibility that this acid treatment might be liberating phenoxy acid or phenol which was bound physically or chemically to natural constituents of milk. The results indicate that no binding of either phenoxy acid or phenol occurred (Table VI). A similar conclusion was reached for 2,4-D (Yip and Ney, 1966).

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LITERATURE CITED

Bache, C. A., Hardee, D. D , Lisk, D J., *J. Dairy Sci.* **47,** 298 (1964).
DeBoer, T. J., Backer, H. J , "Organic Syntheses," Collect. Vol. IV, N. Rabjohn, Ed., Wiley, New York, N.Y., 1963, p 250.
Gutenmann, W. H., Hardee, D. D., Holland, R. F., Lisk, D. J., *J. Dairy Sci.* **46,** 991 (1963a).
Gutenmann, W. H., Hardee, D. D., Holland, R. F., Lisk, D. J., *J. Dairy Sci.* **46,** 1287 (1963b).
Klingman, D. L., Gordon, C. H., Yip, G., Burchfield, H. P., *Weeds* **14,** 164 (1966).
Kutschinski, A. H., J. AGR. FOOD CHEM. **17,** 288 (1969).
St. John, L. E., Wagner, D. G., Lisk, D. J., *J. Dairy Sci.* **47,** 1267 (1964).
Yip, G., Ney, R. E., *Weeds* **14,** 167 (1966).

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Determination of Ethylenethiourea Residues in Apples

William H. Newsome

Ethylenethiourea was determined in apples in the 0.01–1.00 ppm range with an overall recovery of 94.7 ± 5.9%. The procedure involves conversion to the *S*-benzyl derivative, followed by extraction, trifluoroacetylation, and quantitative measurement by gas–liquid chromatography. The cleanup is rapid and effective. Specific confirmation of the derivative was carried out by mass spectrometry. Commercial samples were found to contain 0.018–0.044 ppm of ethylenethiourea.

Ethylenethiourea (ETU) has been reported as a decomposition product of a widely used group of fungicides, the ethylenebisdithiocarbamates (Ludwig *et al.*, 1954; Czegledi-Janko, 1967). The compound has also been described as tumorigenic in mice (Innes *et al.*, 1969). Reports in the literature vary as to whether ETU is accumulated by plants (Ross and Ludwig, 1957; Vonk and Kaars Sijpesteijn, 1970) or is rapidly dissipated (Yip *et al.*, 1971). Because of the potential health hazard, methods are required for determining the residue on or in foods.

ETU is highly polar, being soluble in water or lower alcohols and making extraction without accompanying interfering substances difficult. Further, the unmodified compound was found unsuitable for gas–liquid chromatography. A recently described method for ETU (Onley and Yip, 1971) is highly sensitive but requires extensive cleanup and is not amenable to rigorous confirmation. The analytical procedure presented here involves conversion of ETU to a known compound, *S*-benzyl ETU, and cleanup by solvent extraction followed by trifluoroacetylation and quantitation by gas–liquid chromatography. Precise confirmation of the derivative has been carried out by combined glc–mass spectrometry.

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EXPERIMENTAL

Materials. 2-Imidazolidenethione (ETU) was purchased from Eastman Organic Chemicals, Rochester, N. Y., and was recrystallized twice from 95% ethanol to give a white crystalline product, mp 201–203°. The recrystallized ETU was dissolved in water and added to samples of apple in volumes of less than 0.50 ml.

The *S*-benzyl ETU used as a reference standard was pre-